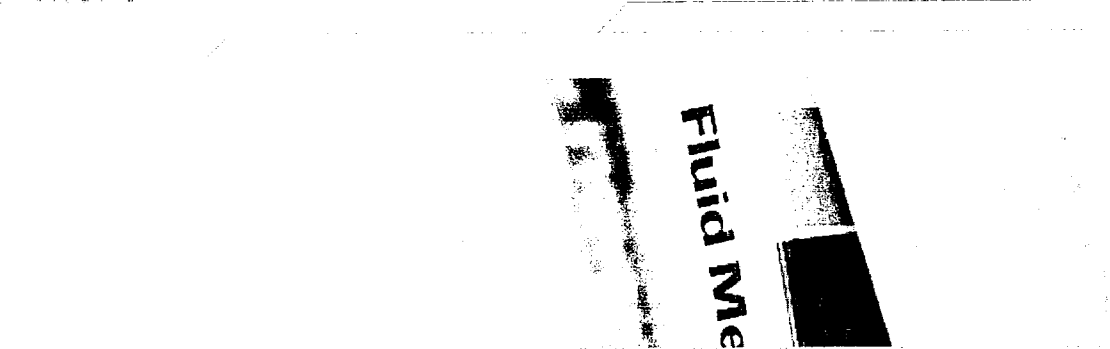


CAT.INIST

Le CNRS, Cote INIST, A 1000

**Titre du document / Document title**

An alternative mechanism of bioluminescence color determination in firefly luciferase

**Auteur(s) / Author(s)**

BRANCHINI Bruce R. ; SOUTHWORTH Tara L. ; MURTIASHAW Martha H. ; MAGYAR Rachelle A. ; GONZALEZ Susan A. ; RUGGIERO Maria C. ; STROH Justin G. ;

**Résumé / Abstract**

Beetle luciferases (including those of the firefly) use the same luciferin substrate to naturally display light ranging in color from green ( $\lambda_{\text{max}} \sim 530$  nm) to red ( $\lambda_{\text{max}} \sim 635$  nm). In a recent communication, we reported (Branchini, B. R., Murtiashaw, M. H., Magyar, R. A., Portier, N. C., Ruggiero, M. C., and Stroh, J. G. (2002) *J. Am. Chem. Soc.* 124, 2112-2113) that the synthetic adenylate of firefly luciferin analogue D-5,5-dimethyluciferin was transformed into the emitter 5,5-dimethyloxyluciferin in bioluminescence reactions catalyzed by luciferases from *Photinus pyralis* and the click beetle *Pyrophorus plagiophthalmus*. 5,5-Dimethyloxyluciferin is constrained to exist in the keto form and fluoresces mainly in the red. However, bioluminescence spectra revealed that green light emission was produced by the firefly enzyme, and red light was observed with the click beetle protein. These results, augmented with steady-state kinetic studies, were taken as experimental support for mechanisms of firefly bioluminescence color that require only a single keto form of oxyluciferin. We report here the results of mutagenesis studies designed to determine the basis of the observed differences in bioluminescence color with the analogue adenylate. Mutants of *P. pyralis* luciferase putative active site residues Gly246 and Phe250, as well as corresponding click beetle residues Ala243 and Ser247 were constructed and characterized using bioluminescence emission spectroscopy and steady state kinetics with adenylate substrates. Based on an analysis of these and recently reported (Branchini, B. R., Southworth, T. L., Murtiashaw, M. H., Boije, H., and Fleet, S. E. (2003) *Biochemistry* 42, 10429-10436) data, we have developed an alternative mechanism of bioluminescence color. The basis of the mechanism is that luciferase modulates emission color by controlling the resonance-based charge delocalization of the anionic keto form of the oxyluciferin excited state.

**Revue / Journal Title**

Biochemistry (Biochemistry) ISSN 0006-2960

**Source / Source**

2004, vol. 43, n°23, pp. 7255-7262 [8 page(s) (article)]

**Langue / Language**

Anglais

**Editeur / Publisher**

American Chemical Society, Washington, DC, ETATS-UNIS (1962) (Revue)

**Localisation / Location**

INIST-CNRS, Cote INIST : 9758, 35400011363786.0040

**Copyright 2007 INIST-CNRS. All rights reserved**

Toute reproduction ou diffusion même partielle, par quelque procédé ou sur tout support que ce soit, ne pourra être faite sans l'accord préalable écrit de l'INIST-CNRS.

No part of these records may be reproduced or distributed, in any form or by any means, without the prior written permission of INIST-CNRS.

N° notice reldoc (ud4) : 15864633